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Retention in Treated Wastewater Affects Survival and Deposition of *Staphylococcus aureus* and *Escherichia coli* in Sand Columns

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The fate and transport of pathogenic bacteria from wastewater treatment facilities in the Earth's subsurface have attracted extensive concern over recent decades, while the impact of treated-wastewater chemistry on bacterial viability and transport behavior remains unclear. The influence of retention time in effluent from a full-scale municipal wastewater treatment plant on the survival and deposition of *Staphylococcus aureus* and *Escherichia coli* strains in sand columns was investigated in this paper. In comparison to the bacteria cultivated in nutrient-rich growth media, retention in treated wastewater significantly reduced the viability of all strains. Bacterial surface properties, e.g., zeta potential, hydrophobicity, and surface charges, varied dramatically in treated wastewater, though no universal trend was found for different strains. Retention in treated wastewater effluent resulted in changes in bacterial deposition in sand columns. Longer retention periods in treated wastewater decreased bacterial deposition rates for the strains evaluated and elevated the transport potential in sand columns. We suggest that the wastewater quality should be taken into account in estimating the fate of pathogenic bacteria discharged from wastewater treatment facilities and the risks they pose in the aquatic environment.

The primary goals of wastewater treatment plants are to safeguard the quality of the water environment and to conserve water resources. The effluent from wastewater treatment plants is either discharged into receiving watersheds or reused in the agricultural and industrial sectors, such as agricultural irrigation, groundwater recharge, etc. Owing to the huge volumetric amount of treated wastewater, the transport and fate of pathogenic microorganisms from treated wastewater in subsurface and groundwater have attracted extensive concern over the past 2 decades (1–4).

Many studies have led to our increased understanding of the mechanisms of bacterial transport through the Earth's subsurface and contribute to controlling it. Processes influencing the deposition and transport of pathogenic bacteria can be of a physicochemical and biological nature. The physicochemical factors governing bacterial deposition in aquatic systems, such as surface charge characteristics (5, 6), hydrophobic interactions (7), surface macromolecules (8–11), solution chemistry (12–14), and hydrodynamic conditions (15, 16), have been extensively investigated. Biological processes that influence the transport of bacteria are governed by properties inherent in the microbial component of the system, including the growth phase (17, 18), starvation (19), motility (12), and metabolic activity (20, 21). As it is specifically relevant in environmental applications, bacterial transport has been studied in subsurfaces and groundwater. For instance, interactions of the traveling organisms with soil, sand, gravel, or other model granular materials have been conducted using laboratory-scale packed-bed columns under well-controlled environmental conditions (10, 12).

Within the current body of literature, little attention has been paid to the fate of waterborne pathogenic microbes under nutrient-deficient conditions that prevail in the natural environment and wastewater treatment facilities (17, 22–24). Walker reported that, for both *Burkholderia cepacia* G4g and ENV435g, the deposition rates for cells cultivated in nutrient-rich medium differed from those in nutrient-poor basal salt medium (17). In addition, Marcus et al. investigated the effect of the growth solution on the

cell surface properties and transport behavior of 11 *Escherichia coli* isolates, and they observed significant differences in cell surface properties and transport behavior for some cells according to the growth solution (24). However, to our knowledge, no such studies have been performed on bacterial deposition and transport in aqueous chemistry relevant to treated wastewater. Pathogens originating from the fecal wastes of humans or animals have been exposed to low-nutrient conditions in drainage pipes and wastewater treatment plants for some amount of time before they reach the subsurfaces. Since bacteria are inherently dynamic organisms and their surface properties, e.g., protein coverage and macromolecular conformation, evolve with changing physicochemical environments and nutrient concentrations, the retention time in treated wastewater affects the bacterial cell characteristics and potentially their deposition and transport behavior.

The main goal of this research is to examine the effect of retention of pathogenic bacteria in a real effluent from a full-scale municipal wastewater treatment plant on survival and bacterial deposition in quartz sand packed-bed columns. In this paper, Gram-positive *Staphylococcus aureus* and Gram-negative *E. coli* strains were selected as model systems because they are ubiquitous

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in hospital facilities and wastewater treatment plants. The surface properties and deposition of different strains in treated wastewater were determined. The mechanisms controlling bacterial transport were also proposed in this study.

MATERIALS AND METHODS

Wastewater collection. Treated wastewater was collected from the discharge outlet of a municipal wastewater treatment plant with conventional activated sludge and tertiary sand filtration treatment processes (Gaobeidian municipal wastewater treatment plant, Beijing, China). The quality of the treated wastewater is shown in Table S1 in the supplemental material. Sterile wastewater was obtained by filtering through a 0.22- μm filter (Millipore Purapore polyvinylidene difluoride [PVDF] membrane) and stored at 4°C.

Bacterial strains and growth conditions. *S. aureus* ATCC 6538P, *E. coli* D21, and enterotoxigenic *E. coli* (ETEC) O126:K71 were involved in this study. The growth media for *S. aureus* and *E. coli* strains were tryptone soya broth (TSB) (Oxoid, Basingstoke, England) and Luria broth (LB) (tryptone, 10 g/liter; yeast extract, 5 g/liter; NaCl, 10 g/liter; pH 7.0), respectively. Each strain was routinely cultured aerobically at 37°C on a TSB/LB agar plate. One colony was used to inoculate 10 ml TSB/LB, and this preculture was grown for 24 h at 37°C. The preculture was diluted 1:20 in 200 ml TSB/LB and grown for 16 h at 37°C. Bacteria were pelleted by centrifugation (3K15; Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) for 5 min at $5,000 \times g$ in a 12158H rotor and resuspended in 10 mM KCl solution. Centrifugation was done twice in order to remove all traces of growth medium. To break staphylococcal aggregates, sonication at 30 W (Scientz JY96-II; Ningbo Scientz Biotechnology, Co., Ltd., China) was applied (3 times for 10 s each time) during cooling in an ice-water bath. The hydrodynamic diameter of the staphylococci was 1.1 μm on average, as determined using dynamic light scattering. The equivalent spherical diameters of the *E. coli* D21 and ETEC O126:K71 cells were determined to be 1.6 and 1.5 μm , respectively.

Incubation of bacteria in treated wastewater. The bacterial cells were added to sterile wastewater to a concentration of 3×10^8 cells/ml, as determined in a Bürker-Türk counting chamber, and subsequently incubated at 37°C for 24, 96, or 192 h. The bacteria obtained directly from the growth medium, i.e., TSB or LB, without incubation in wastewater were designated the ones with a retention time of 0 h. The concentration of viable bacteria was evaluated by counting the CFU on TSB/LB agar plates after proper dilution in sterile phosphate-buffered saline (PBS). The percentage of survivors is expressed as the ratio of the concentration of viable cells in treated wastewater for a designated retention time to that at 0 h retention. After the desired incubation period, the cells were pelleted again and suspended in 10 mM KCl solution. This suspension was used in subsequent bacterial surface characterization, transport experiments, and electron microscopy.

Bacterial electrophoretic mobility, hydrophobicity, and surface acidity. The electrophoretic mobility of the bacterial cells was determined by diluting the bacterial suspension in a KCl electrolyte solution with varying ionic strengths from 1 to 100 mM at a final concentration of 10^5 to 10^6 cells/ml. Electrolyte solutions were prepared with deionized water and KCl at pH 7.0 ± 0.1 . Electrophoretic mobility measurements were conducted at 25°C using a zeta analyzer (Zetasizer nano Z; Malvern Instruments Ltd., Worcestershire, United Kingdom) and were repeated three times at each ionic strength. The hydrophobicity of bacterial cells was measured using the microbial adhesion to hydrocarbons test with *n*-dodecane (25). Potentiometric titrations were conducted to determine the relative acidities of the bacterial surfaces using a microtitrator (794 Basic Titrimo; Metrohm, Herisau, Switzerland). The bacterial suspension was diluted in 10 mM KCl to a concentration of 3×10^8 cells/ml. Prior to titration, the pH of the newly prepared suspension was lowered to 4 by addition of HCl, and N_2 gas was introduced into the suspension to remove any dissolved CO_2 present. Then, titrations were performed with an NaOH solution under a nitrogen atmosphere. The acidity was determined

from the amount of NaOH consumed, which provides a measure of the total number of charged functional groups on the outer envelope of the bacterial cell.

Transmission electron microscopy (TEM). Bacteria were harvested at the desired retention time in sterile treated wastewater and then fixed via a glutaraldehyde-osmium tetroxide protocol, dehydrated using an ethanol-propylene oxide series, and embedded in LR White (London Resins, Marivac, Nova Scotia, Canada) according to a method described previously (26). Thin sections were stained with uranyl acetate and lead citrate. Imaging was performed using an FEI CM-100 transmission electron microscope (FEI Corp., Hillsboro, OR, USA) operating at 100 kV under standard conditions.

Sand column transport experiments. Bacterial transport in sand columns followed the protocol reported by Redman et al. (27). High-purity quartz sand grains were used in the packed-bed column transport experiments. Sieve analysis resulted in an average grain diameter (d_{50}) of 205 μm and a coefficient of uniformity (d_{60}/d_{10} [where d_{60} is the size of the sieve at which 60% of the sample weight passes and d_{10} is the size of the sieve at which 10% of the sample weight passes]) of 1.26. The porosity of the quartz sand was determined to be 0.43 by measuring the pore volume of interstitial liquid in the sand packed bed. The sieved quartz sand was treated to remove surface impurities according to a method reported previously (26). Briefly, the sand was soaked in 12 N HCl for at least 24 h, washed in deionized water, and finally baked at 800°C for a minimum of 8 h. The clean quartz sand was dried in an oven at 80°C and then stored in high-density polyethylene containers until use.

To measure the zeta potentials of the quartz sands, the sand particles were ground manually in a mortar for 10 min, and the colloidal portion of the sand particles was used to perform the zeta potential measurements using a zeta analyzer (Zetasizer nano Z; Malvern Instruments Ltd., Worcestershire, United Kingdom) in KCl electrolyte solutions with varying ionic strengths.

Bacterial-transport experiments were conducted in glass chromatography columns (1-cm inner diameter) packed with clean quartz sand grains. The bacterial suspension was diluted in KCl solutions with ionic strengths varying from 1 to 100 mM to a concentration (C_0) of 3×10^8 cells/ml. The columns were equilibrated with more than 20 pore volumes of bacterium-free KCl solutions with ionic strengths identical to that of the bacterial suspension. Peristaltic pumps were used to regulate the flow, and the superficial fluid velocity was maintained at 0.021 cm/min. Following the equilibration step, the bacterial suspension was injected into the columns, and the injection lasted for 15 min (approximately 4 pore volumes). The concentration of the bacterial cells in the column effluent, C , was continuously monitored every 30 s using a spectrophotometer by measuring the optical density at a wavelength of 546 nm. Upon the termination of cell suspension injection, the columns were flushed with bacterium-free KCl solution for at least 45 min (approximately 12 pore volumes).

The kinetics of the deposition of bacterial cells within the saturated sand columns could be estimated by calculating the deposition rate coefficient, k_d (1/min), from the early cell breakthrough concentrations in the effluent (27) as follows: $k_d = (U/fL) \ln(C/C_0)$, in which f is the porosity of the sand (cm^3/cm^3), U is the approach fluid velocity (cm/min), L is the length of the column (cm), and C/C_0 is the normalized breakthrough concentration, which was obtained from average bacterial breakthrough concentrations between 1.8 and 2.0 pore volumes.

To determine the attachment efficiency of bacterial cells approaching the quartz sand, transport experiments were performed under favorable (nonrepulsive) electrostatic conditions (27). To produce a favorable surface, the quartz was silanized by suspending the sand in a 1% solution of 3-aminopropyl-triethoxysilane (Sigma-Aldrich, Co. LLC, St. Louis, MO, USA) for 5 min, followed by thorough rinsing in deionized water and curing at 80°C for 24 h. The zeta potential of the silanized sand was measured to confirm the presence of a favorable surface. The deposition rates in the silanized sand columns were determined and used as the favorable

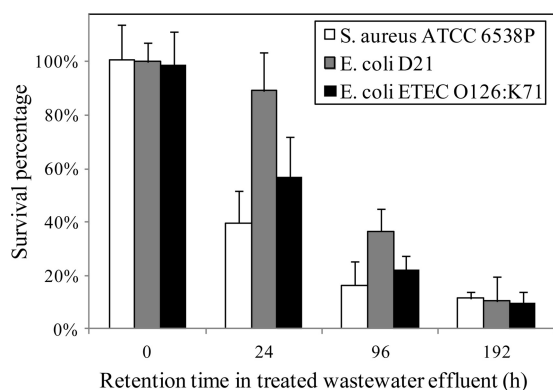


FIG 1 Survival percentages of *S. aureus* ATCC 6538P, *E. coli* D21, and ETEC O126:K71 at different retention times in treated wastewater. The survival percentage is expressed as the ratio of viable bacterial cells at a designated retention time to that at 0 h in treated wastewater. The error bars represent standard deviations.

deposition rate ($k_{d,fav}$). Thus, the attachment efficiency, α_{att} , can be calculated as follows: $\alpha_{att} = k_d/k_{d,fav}$.

Enzyme treatment of EPS. To investigate the role of extracellular polymeric substances (EPS) in *S. aureus* ATCC 6538P deposition in a sand column, a cellulase from *Trichoderma reesei* ATCC 26921 (Sigma-Aldrich) was employed to partially cleave the EPS on the cell envelope according to a method described previously (28) with minor modifications. Briefly, *S. aureus* ATCC 6538P cells incubated in treated wastewater for 192 h were harvested by centrifugation and resuspended in 10 mM KCl to 2×10^8 cells/ml, and the enzyme cellulase was added (4 mg/ml). The suspension was agitated at 50 rpm for 1 h on a horizontal shaker table (25°C). The treated cells were again washed twice with 10 mM KCl solution and harvested for sand column transport experiments.

Statistics. Data were statistically analyzed using paired two-tailed Student *t* tests. Significance was established at a *P* value of <0.05 .

RESULTS

Retention in treated wastewater significantly affected the survival percentages of *S. aureus* and *E. coli* strains, as shown in Fig. 1. For *S. aureus* ATCC 6538P, the viable bacterial cells decreased rapidly to about 40% of their initial concentration after 24 h of incubation in treated wastewater, and only 11% of the cells could survive 192 h of retention. Also, *E. coli* D21 and ETEC O126:K71 viability decreased with increasing time in treated wastewater. Twenty-four hours of retention in treated wastewater led to a higher survival percentage of *E. coli* D21 (88%) than of ETEC O126:K71

(57%). Nevertheless, longer retention times resulted in similar survival percentages for both *E. coli* strains, and only 10% of the bacterial cells remained viable.

The influence of ionic strength on the zeta potentials of the bacteria with varying retention times in treated wastewater is presented in Fig. 2. The results indicate that all the bacteria were negatively charged over the range of ionic strengths and pH conditions tested. For *S. aureus* ATCC 6538P, a 24-h retention in treated wastewater led to more negative zeta potentials at the ionic strengths of 1 and 10 mM KCl in comparison to the bacteria cultivated in TSB medium, while longer retention times, e.g., 96 and 192 h, resulted in less negative zeta potentials (Fig. 2a). For both *E. coli* strains, on the other hand, retention in treated wastewater did not produce significant changes in the zeta potential (Fig. 2b and c).

Potentiometric titration results are shown in Table S2 in the supplemental material as the bacterial surface acidity, which indicated the amount of NaOH consumed by the suspended cells titrated between pH values of 4 and 10. The acidities of *S. aureus* and *E. coli* bacteria cultivated in nutrient-rich media varied between 7.32×10^{-5} and 1.35×10^{-4} meq per 10^8 cells. Retention in treated wastewater produced adverse influences on the bacterial surface acidity for *S. aureus* and *E. coli* strains. Retention of *S. aureus* ATCC 6538P in treated wastewater significantly increased the bacterial surface acidity. On the other hand, retention in treated wastewater decreased the surface acidity of *E. coli* strains.

Retention in treated wastewater resulted in a significant reduction in the hydrophobicity of *S. aureus* ATCC 6538P, as shown in Fig. 3. The hydrophobicity value of *S. aureus* ATCC 6538P cultivated in TSB medium was 75.7%, while the values changed drastically to 7.2%, 15.2%, and 16.1% for the bacteria with retention times of 24, 96, and 192 h in treated wastewater, respectively. For both *E. coli* strains, retention in treated wastewater significantly increased hydrophobicity in comparison with the bacterial cells cultivated in LB medium. A retention time of 24 h in treated wastewater elevated their hydrophobicity, while hydrophobicity remained statistically unchanged with further increases in the retention time.

The transport of *S. aureus* and *E. coli* bacteria was systematically examined in saturated and negatively charged (see Fig. S1 in the supplemental material) quartz grains. Typical breakthrough curves demonstrating the effects of ionic strength and retention time in treated wastewater on bacterial transport are shown in Fig. 4 and 5 and in Fig. S2 in the supplemental material. In the figures,

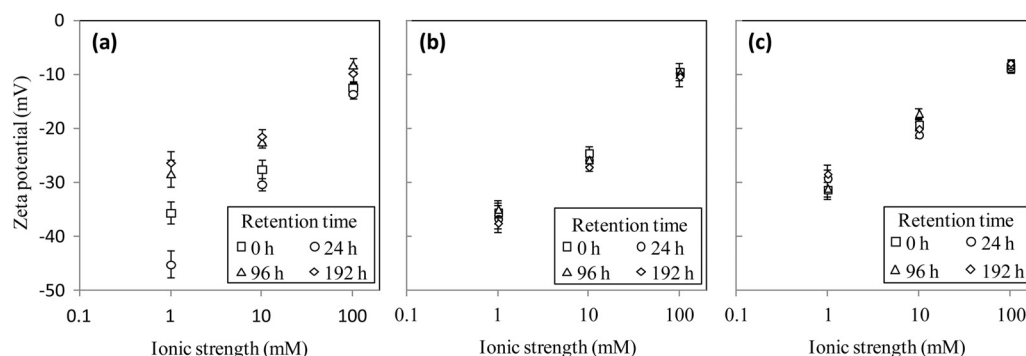


FIG 2 Zeta potentials of *S. aureus* ATCC 6538P (a), *E. coli* D21 (b), and *E. coli* ETEC O126:K71 (c) at different retention times in treated wastewater. The error bars represent standard deviations.

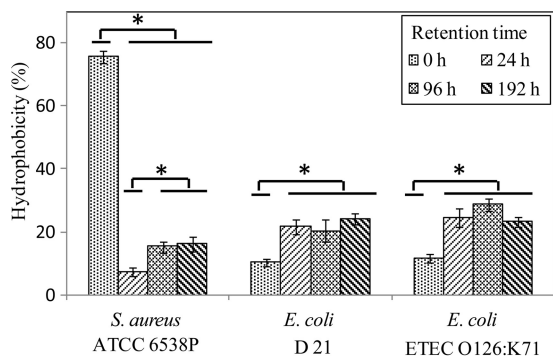


FIG 3 Hydrophobicities of different bacteria at different retention times in effluent from a municipal wastewater treatment plant. The error bars represent standard deviations, and asterisks indicate significant differences.

the normalized effluent concentration is plotted against the number of pore volumes passed through the column. After approximately 1 pore volume, the injected bacterial cells broke through the packed bed and were detected in the effluent. The influent was switched to a bacterium-free solution after approximately 4 pore volumes, and the concentration of bacteria in the effluent decreased. The impacts of retention in treated wastewater on bacterial transport differed significantly for different strains tested in the experiment.

For *S. aureus* ATCC 6538P, an increase in the ionic strength of the fluid resulted in larger fractions of bacterial deposition onto the quartz grain surface, as shown in Fig. 4. At an ionic strength of 1 mM KCl, the transport trends correlated well with the results of zeta potential measurements (Fig. 4a). When the ionic strength increased to 10 mM KCl, larger fractions of injected bacteria were present in the effluent of the packed columns for bacteria incubated in treated wastewater for 96 and 192 h than for their more highly negatively charged counterparts in TSB medium (Fig. 4b). Under 100 mM ionic-strength conditions, about 32% of injected bacterial cells were present in the effluent of packed-bed columns for *S. aureus* ATCC 6538P retained in treated wastewater for 192 h, while nearly all injected bacteria were retained for bacteria at retention times of 0, 24, and 96 h (Fig. 4c).

For *E. coli* D21, at an ionic strength of 1 mM KCl, larger fractions of injected bacteria with retention times of 0 and 24 h in treated wastewater were retained in the packed columns, significantly larger than for the bacteria with longer retention times in treated wastewater (Fig. 5a). When the ionic strength of the fluid

in the packed bed was 10 mM KCl, the bacteria with a retention time of 192 h had the largest deposition fraction (Fig. 5b). Under an ionic strength of 100 mM KCl, the bacteria in treated wastewater for 0 and 24 h showed lower tendencies for deposition than their counterparts with longer retention times (Fig. 5b).

ETEC O126:K71 exhibited much higher deposition rates in the sand columns (see Fig. S2 in the supplemental material), and comparatively low concentrations of bacterial cells (less than 10% of C_0) were detected in the effluent under all ionic-strength conditions. Higher deposition rates in the sand column were observed with increasing ionic strength. The cells with longer retention times (96 or 192 h) in treated wastewater showed a slightly higher tendency for transport from the sand columns.

DISCUSSION

Pathogen survival in wastewater and groundwater has been extensively studied. Some studies reported that the times required to reduce the bacterial population by 1 order of magnitude for various strains in groundwater ranged from 2 to 6 days (29, 30). Our results displayed similar decay rates, and almost 10% of *S. aureus* and *E. coli* strains were viable after 8 days of retention in treated wastewater. Most bacteria from wastewater can survive in an aquatic environment for weeks to months, but their numbers are greatly reduced during exposure to the environment.

The bacterial cell surface is highly dynamic, responding strongly to environmental changes. In comparison to nutrient-rich media, such as TSB and LB, treated wastewater contains much lower levels of organic carbon, nitrogen, and phosphorus sources needed for bacterial growth (see Table S1 in the supplemental material), which may alter the composition, conformation, and electric charges of bacterial surface macromolecules. In the present study, retention in treated wastewater resulted in significant changes in the surface acidities, zeta potentials, and hydrophobicities of different bacterial strains. Sanin et al. also reported that the surface hydrophobicities of *Pseudomonas* sp. and *Rhodococcus corallines* strains stayed constant under carbon starvation conditions while significantly decreasing when the bacteria were starved for nitrogen (31). Haznedaroglu et al. reported that starvation of two *E. coli* strains isolated from human and cattle waste in nutrient-free KCl solution led to significant changes in the bacterial surface charge densities, hydrophobicities, and zeta potentials (19).

The changes in bacterial surface properties also altered transport behavior in quartz sand columns in terms of the deposition rate coefficients, k_d , and attachment efficiencies, α_{att} (Fig. 6; see Fig. S3 in the supplemental material), albeit the impacts are dif-

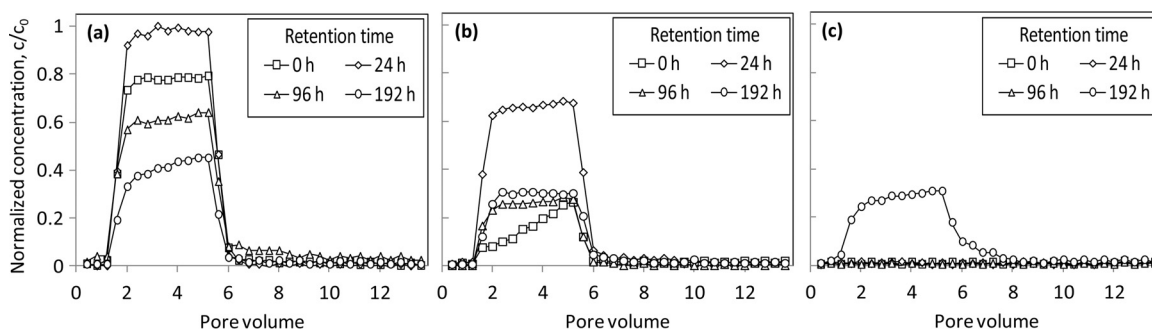


FIG 4 Breakthrough curves of *S. aureus* ATCC 6538P in quartz sand columns in 1 mM (a), 10 mM (b), and 100 mM (c) KCl solutions for different retention times in treated wastewater.

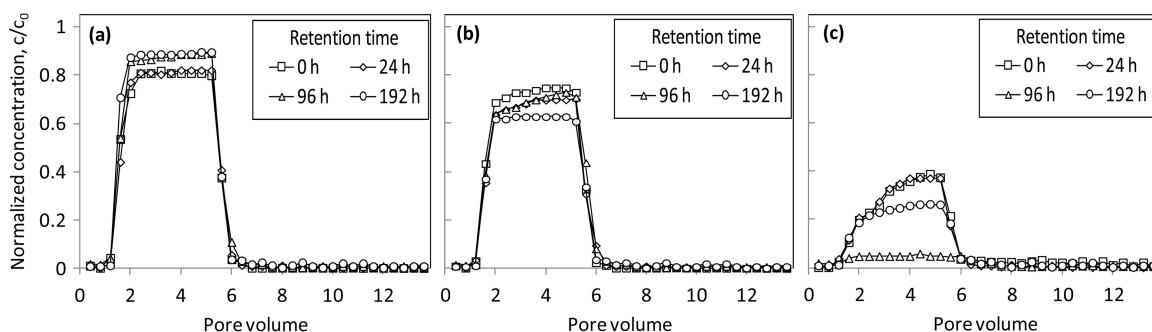


FIG 5 Breakthrough curves of *E. coli* D21 in quartz sand columns in 1 mM (a), 10 mM (b), and 100 mM (c) KCl solutions at different retention times in treated wastewater.

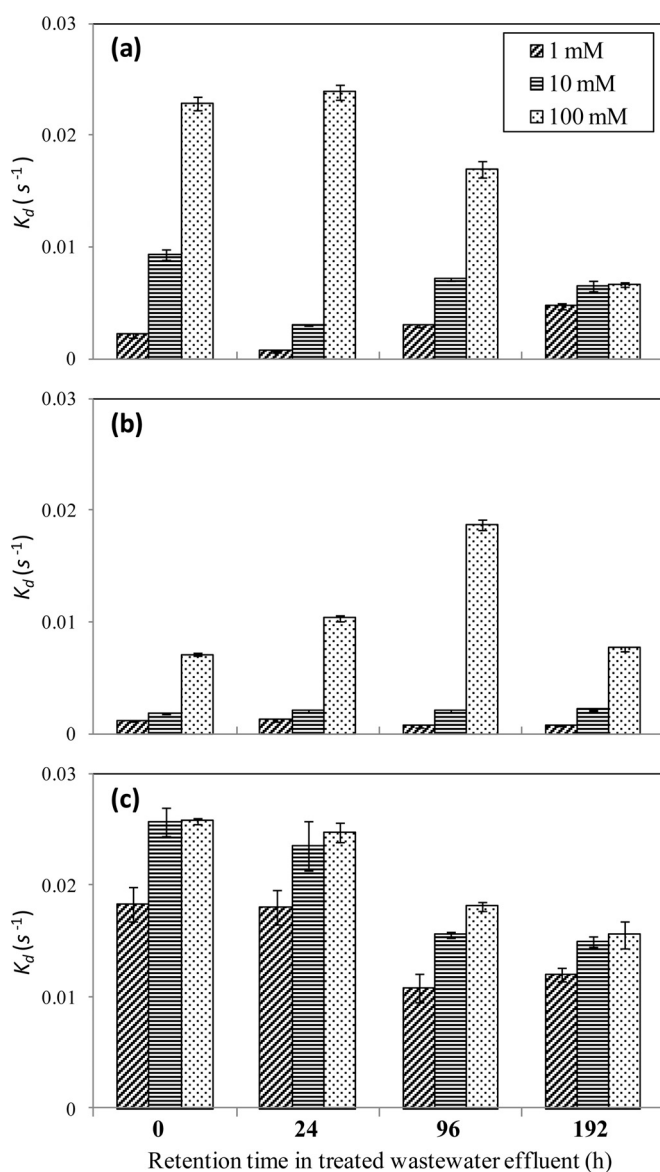


FIG 6 Deposition rate coefficients of *S. aureus* ATCC 6538P (a), *E. coli* D21 (b), and ETEC O126:K71 (c) in cleaned sand columns as a function of retention time in effluent from a municipal wastewater treatment plant. The error bars represent standard deviations.

ferent for bacterial strains. In most experiments, a higher ionic strength of the pore fluid resulted in higher k_d and α_{att} values, except for *S. aureus* ATCC 6538P in treated wastewater for 192 h.

Bacterial transport in sand columns has usually been described by the DLVO (Derjaguin, Landau, Verwey, and Overbeek) theory (32, 33). The total interaction energy, namely, the sum of repulsive electrostatic interactions and attractive van der Waals forces, was calculated by modeling the bacterium-quartz grain system with a sphere-plate interaction. Repulsive electrostatic double-layer interaction energies were calculated using the Hogg et al. (34) expression:

$$\Phi_{EDL} = \pi \epsilon_0 \epsilon_r a_p \left\{ 2\psi_p \psi_c \ln \left[\frac{1 + \exp(-\kappa h)}{1 - \exp(-\kappa h)} \right] + (\psi_p^2 + \psi_c^2) \ln[1 - \exp(-2\kappa h)] \right\},$$

where ϵ_0 is the dielectric permittivity in a vacuum, ϵ_r is the relative dielectric permittivity of water, a_p is the bacterial radius, κ is the inverse Debye length, h is the separation distance between the bacterium and the collector surface, and ψ_p and ψ_c are the surface potentials of the bacterial cell and quartz collector, respectively. The van der Waals attractive interaction energy was calculated from the following equation: $\Phi_{VDW} = -(Aa_p/6h)[1 + (14h/\lambda)]$, where A is the Hamaker constant of the interacting media (bacteria-water-quartz) and λ is the characteristic wavelength of the dispersion interaction. The values of A and λ were selected from Redman et al. (27) as 6.5×10^{-21} J and 100 nm, respectively, owing to similar experimental conditions.

A typical DLVO interaction energy as a function of the separation distance and ionic strength for *S. aureus* ATCC 6538P in TSB medium was present (Fig. 7). The bacterium-sand DLVO interaction parameters for different strains with varying retention times in treated wastewater are listed in Table S3 in the supplemental material. As shown for solutions with 1 and 10 mM, calculations predict the presence of a substantial repulsive energy barrier to bacterial deposition ranging from 320 kT to 1,983 kT (where k is Boltzmann's constant and T is absolute temperature). In 100 mM KCl, no energy barrier existed for all bacteria. The huge energy barriers suggested that it is unlikely that bacterial cells would be deposited in the primary energy minimum at the quartz surface. However, DLVO calculations predicted the presence of a secondary energy minimum at a much greater separation distance than that of the energy barrier (Fig. 7b; see Table S3 in the supplemental material), which means that bacteria approaching a quartz grain

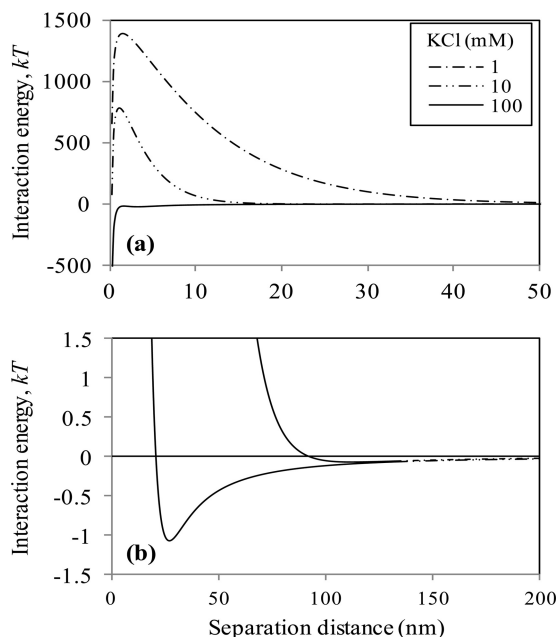


FIG 7 Typical DLVO interaction energy as a function of separation distance and ionic strength for *S. aureus* ATCC 6538P (a) to highlight the secondary energy minimum in 1 mM and 10 mM KCl solution (b). The Hamaker constant in the bacteria-water-quartz sand system is 6.5×10^{-21} J.

would first experience an attractive force before encountering the repulsive energy barrier. The magnitudes of the secondary energy minimum for different bacteria range from 0.07 kT at 1 mM to around 1.21 kT at 10 mM, with corresponding separation distances of around 120 and 20 nm, respectively. In previous research, the secondary minimum in DLVO interaction energy was suggested to play an important role in the deposition of bacterial cells in packed-bed columns under unfavorable electrostatic conditions (27).

If the hydrophobicity was also taken into account, bacterial deposition in 1 mM KCl was in good agreement with the DLVO predictions. For instance, *S. aureus* ATCC 6538P in treated wastewater for 24 h had the highest negative charge (Fig. 3a), and consequently, the lowest magnitudes of k_d were measured (Fig. 6a). Similar zeta potentials and higher hydrophobicity for *E. coli* D21 with increasing retention times of 24, 96, and 192 h led to significant reductions in bacterial deposition rate coefficients on hydrophilic quartz sand surfaces (Fig. 6b). Under moderate- and high-ionic-strength conditions, the bacterial transport trends contradicted the DLVO predictions, especially for those bacteria with longer retention times in treated wastewater. In the literature, many studies have reported that different *E. coli* strains changed the compositions and structures of cell surface macromolecules in response to the alterations of fluid chemistry and nutrient levels (19, 35, 36). In this study, we found that longer retention times in treated wastewater induced the production of EPS with a thickness up to 20 to 30 nm on the outside of *S. aureus* ATCC 6538P cells, as depicted in Fig. S4 in the supplemental material. The influence of EPS on bacterial deposition under high-ionic-strength conditions has been observed by others, as well (37). They found that bacterial deposition was dominated by steric interaction between the outer cell surface macromolecules and the substrata at

an ionic strength higher than 0.1 M. In addition, the presence of the EPS layer might change the electrophoretic properties of bacterial cells. Ohshima proposed a so-called soft-layer model to interpret the electrophoretic mobility of soft, polyelectrolyte-covered particles as opposed to rigid particles (38, 39). In the soft-layer analysis of electrophoretic mobility, this is expressed in the softness parameter (38). Based on our experimental results, *S. aureus* ATCC 6538P grown in TSB medium was relative hard owing to its comparatively rigid peptidoglycan-rich outer surface, while the bacterial cells became soft (softness parameter, 2.8 nm) as they were retained in treated wastewater for 192 h.

To address the influence of this EPS layer on bacterial deposition, cellulase was used to remove the extracellular polysaccharides that are major components of EPS (40). After cellulase treatment (see Fig. S4c in the supplemental material), the bacterial cells became less negatively charged and more hydrophilic (data not shown), and consequently, a larger fraction of injected bacteria were deposited in the packed-bed columns (see Fig. S5 in the supplemental material).

In summary, the viability of *S. aureus* and *E. coli* bacteria decreased significantly with increasing retention times in the effluent of a full-scale wastewater treatment plant. Bacterial surface properties, such as zeta potential, hydrophobicity, and charge density, varied as a function of the retention time, but no universal trend was found for different strains. In comparison to the bacterial cells incubated in nutrient-rich media, longer retention periods in treated wastewater reduced bacterial deposition rates in packed-bed sand columns. These results clearly imply that wastewater quality should be taken into account in estimating the fate of bacteria discharged from wastewater treatment facilities and the risks they pose in the aquatic environment.

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